

➤ On Page 16, please replace the paragraph at lines 14-22 with the following text:

B2
Figure 5A is a "pileup" alignment of predicted amino acid sequences which compares *Drosophila hh* (D-*hh*; SEQ ID No:34), mouse *hh* (M-*Dhh*; SEQ ID No:9; M-*Ihh*; SEQ ID No:10; M-*Shh*; SEQ ID No:11), chicken *hh* (C-*Shh*; SEQ ID No:8), and zebrafish *hh* (Z-*Shh*; SEQ ID No:12). The predicted hydrophobic transmembrane/signal sequences are indicated in italics and the predicted signal sequence processing site is arrowed. The positions of introns interrupting the *Drosophila hh* and M-*Dhh* open reading frames are indicated by arrowheads. All amino acids shared among the six predicted *hh* proteins are indicated in bold. Figure 5B is a sequence alignment of the N-terminal portion of vertebrate *hedgehog* proteins (SEQ ID Nos:48-54), and the predicted degenerate sequence "CON" (SEQ ID No: 41).

1 ➤ On Page 17, please replace the paragraph at lines 6-16 with the following text:

B3
Figures 9A and 9B illustrate the comparison of zebrafish *Shh* (Z-*Shh*) and *Drosophila hh* (*hh*) amino acid sequences (SEQ ID Nos:12 and 34). Figure 9A is an alignment of zebrafish *Shh* and *Drosophila hh* amino acid sequences. Identical amino acids are linked by vertical bars. Dots indicate gaps introduced for optimal alignment. Putative transmembrane/signal peptide sequences are underlined (Kyte and Doolittle (1982) *J Mol Biol* 157:133-148). The position of exon boundaries in the *Drosophila* gene are indicated by arrowheads. The region of highest similarity between Z-*Shh* and *hh* overlaps exon 2. Figure 9B is a schematic comparison of Z-*Shh* and *drosophila hh*. Black boxes indicate the position of the putative transmembrane/signal peptide sequences. relative to the amino-terminus. Sequence homologies were scored by taking into account the alignment of chemically similar amino acids and percentage of homology in the boxed regions is indicated.

➤ On Page 17, please replace the paragraph at lines 17-20 with the following text:

B4
Figure 10 (SEQ ID Nos:37 and 15-17) is an alignment of partial predicted amino acid sequences from three different zebrafish *hh* homologs. One of these sequences corresponds to *Shh*, while the other two define additional *hh* homologs in zebrafish, named *hh(a)* and *hh(b)*. Amino acid identities among the three partial homologs are indicated by vertical bars.

➤ On Page 87, please replace the paragraph at lines 19-36 with the following text:

All standard cloning techniques were performed according to Ausubel et. al. (1989), and all enzymes were obtained from Boehringer Mannheim Biochemicals. Degenerate oligonucleotides corresponding to amino acid residues 161 to 237 of the *Drosophila hedgehog* protein (SEQ ID No:34) (Lee, J.J. et. al., (1992) *Cell* 71: 33-50) were synthesized. These degenerate oligonucleotides, vHH50 (SEQ ID No:18), vHH30 (SEQ ID No:19), and vHH3I (SEQ ID No:20) also contained Eco RI, Cla I, and Xba I sites, respectively, on their 5' ends to facilitate subcloning. The nucleotide sequence of these oligos is given below:

B5 vHH50: 5'-GGAATTCCCAG(CA)GITG(CT)AA(AG)GA(AG)(CA)(AG)I(GCT)IAA-3' (SEQ ID No:18)
vHH30: 5'-TCATCGATGGACCCA(GA)TC(GA)AAICCGC(TC)TC-3' (SEQ ID No:19)
vHH3I: 5'-GCTCTAGAGCTCIACIGCIA(GA)IC(GT)IGC-3' (SEQ ID No:20)

where I represents inosine. Nested PCR was performed by first amplifying chicken genomic DNA using the vHH50 and vHH30 primer pair and then further amplifying that product using the vHH50 and vHH3I primer pair. In each case the reaction conditions were: initial denaturation at 93° C for 2.5 min., followed by 30 cycles of 94° C for 45 s, 50° C for 1 min., 72° C for 1, and a final incubation of 72° C for 5 min. The 220 bp PCR product was subcloned into pGEM7zf (Promega). Two unique clones, pCHA (SEQ ID No:35) and pCHB (SEQ ID No:36) were identified.

➤ On Page 96, please replace the paragraph at lines 24-28 with the following text:

Oligonucleotide sequences are as follows:

lac1: 5'-AGCTGTCGACGCGGCCGCTACGTAGGTTACCGACGTCAAGCTTAGATCTC-3' (SEQ ID No:21)

lac2: 5'-AGCTGAGATCTAAGCTTGACGTCGGTAACCTACGTAGCGGCCGCGTCGAC-3' (SEQ ID No:22)

B6 Sf-1: 5'-GATCGGCCAGGCAGGCCTCGCGATATCGTCACCGCGGTATTCGAA-3' (SEQ ID No:23)

Sf-2: 5'-AGTGCCAGTCGGGGCCCCCAGGGCCGCGCC-3' (SEQ ID No:24)

➤ On Page 97, please replace the paragraph at lines 13-17 with the following text:

Oligonucleotide sequences are as follows:

B7 137: 5'-TACCACAGCGGATGGTTCGG-3' (SEQ ID No:25)